

MEMBRANE ASSOCIATED PROTEINS

This application claims the benefit of Patent Cooperation Treaty International application Ser. No. PCT/US00/22315, filed August 14, 2000, entitled MEMBRANE ASSOCIATED

- 5 PROTEINS, which claims the benefit of U.S. Provisional applications U.S. Ser. No. 60/149,641, filed August 17, 1999, and U.S. Ser. No. 60/164,203, filed November 9, 1999. All of these applications are hereby expressly incorporated by reference herein.

TECHNICAL FIELD

- 10 This invention relates to nucleic acid and amino acid sequences of membrane associated proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative, autoimmune/inflammatory, neurological and gastrointestinal disorders.

BACKGROUND OF THE INVENTION

- 15 Eukaryotic cells are surrounded by plasma membranes which enclose the cell and maintain an environment inside the cell that is distinct from its surroundings. In addition, eukaryotic organisms are distinct from prokaryotes in possessing many intracellular organelle and vesicle structures. Many of the metabolic reactions which distinguish eukaryotic biochemistry from prokaryotic biochemistry take place within these structures. The plasma membrane and the
20 membranes surrounding organelles and vesicles are composed of phosphoglycerides, fatty acids, cholesterol, phospholipids, glycolipids, proteoglycans, and proteins. These components confer identity and functionality to the membranes with which they associate.

Integral Membrane Proteins

- 25 The majority of known integral membrane proteins are transmembrane proteins (TM) which are characterized by an extracellular, a transmembrane, and an intracellular domain. TM domains are typically comprised of 15 to 25 hydrophobic amino acids which are predicted to adopt an α -helical conformation. TM proteins are classified as bitopic (Types I and II) and polytopic (Types III and IV) (Singer, S.J. (1990) Annu. Rev. Cell Biol. 6:247-96). Bitopic proteins span the membrane once while
30 polytopic proteins contain multiple membrane-spanning segments. TM proteins that act as cell-surface receptor proteins involved in signal transduction include growth and differentiation factor receptors, and receptor-interacting proteins such as *Drosophila* pecanex and frizzled proteins, LIV-1 protein, NF2 protein, and GNS1/SUR4 eukaryotic integral membrane proteins. TM proteins also act as transporters of ions or metabolites, such as gap junction channels (connexins) and ion channels,
35 and as cell anchoring proteins, such as lectins, integrins, and fibronectins. TM proteins act as vesicle

organelle-forming molecules, such as calveolins, or as cell recognition molecules, such as cluster of differentiation (CD) antigens, glycoproteins, and mucins.

Many membrane proteins (MPs) contain amino acid sequence motifs that target these proteins to specific subcellular sites. Examples of these motifs include PDZ domains, KDEL, RGD, NGR, and GSL sequence motifs, von Willebrand factor A (vWFA) domains, and EGF-like domains. RGD, NGR, and GSL motif-containing peptides have been used as drug delivery agents in cancer treatments which target tumor vasculature (Arap, W. et al. (1998) Science, 279:377-380). Furthermore, MPs may also contain amino acid sequence motifs, such as the carbohydrate recognition domain (CRD), also known as the C-type lectin domain, that mediate interactions with extracellular or intracellular molecules.

Membrane proteins may also interact with and regulate the properties of the membrane lipids. Phospholipid scramblase, a type II plasma membrane protein, mediates calcium dependent movement of phospholipids (PL) between membrane leaflets. Calcium induced remodeling of plasma membrane PL plays a key role in expression of platelet anticoagulant activity and in clearance of injured or apoptotic cells (Zhou Q. et al. (1997) J. Biol. Chem. 272:18240-18244). Scott syndrome, a bleeding disorder, is caused by an inherited deficiency in plasma membrane PL scramblase function (Online Mendelian Inheritance in Man (OMIM) *262890 Platelet Receptor for Factor X, Deficiency of).

Chemical modification of amino acid residue side chains alters the manner in which MPs interact with other molecules, such as phospholipid membranes. Examples of such chemical modifications to amino acid residue side chains are covalent bond formation with glycosaminoglycans, oligosaccharides, phospholipids, acetyl and palmitoyl moieties, ADP-ribose, phosphate, and sulphate groups.

One function of TM proteins is to facilitate cell-cell communication. The slit proteins are extracellular matrix proteins expressed by cells at the ventral midline of the nervous system. Slit proteins are ligands for the repulsive guidance receptor Robo and thus play a role in repulsive axon guidance (Brose, K. et al. (1999) Cell 96:795-806).

In some cases TM proteins serve as transporters or channels in the cell membrane. For example, the mouse transporter protein (MTP) has four transmembrane domains and resides in an intracellular membrane compartment. MTP can mediate transport of nucleosides in vitro. The role of MTP in the cell may therefore be to transfer nucleosides between the cytosol and the lumen of intracellular organelles (Hogue, D. L. (1996) J. Biol. Chem. 271:9801-9808). The human stomatin-like protein (hSLP-1), expressed primarily in the brain, contains an N-terminal domain similar to the erythrocyte internal membrane protein stomatin, as well as a non-specific lipid transfer protein domain at the C-terminus. hSLP-1 is the human homologue of the *C. elegans* behavioral gene unc-

24, which is believed to be involved in lipid transfer between closely apposed membranes (Seidel, G. and Prohaska, R (1998) *Gene* 225:23-29).

The transmembrane 4 superfamily (TM4SF) or tetraspan family is a multigene family encoding type III integral membrane proteins (Wright, M.D. and Tomlinson, M.G. (1994) *Immunol.*

Today 15:588-594). TM4SF is comprised of membrane proteins which traverse the cell membrane four times. Members of the TM4SF include platelet and endothelial cell membrane proteins, melanoma-associated antigens, leukocyte surface glycoproteins, colonal carcinoma antigens, tumor-associated antigens, and surface proteins of the schistosome parasites (Jankowski, S.A. (1994) *Oncogene* 9:1205-1211). Members of the TM4SF share about 25-30% amino acid sequence identity with one another.

A number of TM4SF members have been implicated in signal transduction, control of cell adhesion, regulation of cell growth and proliferation, including development and oncogenesis, and cell motility, including tumor cell metastasis. Expression of TM4SF proteins is associated with a variety of tumors and the level of expression may be altered when cells are growing or activated.

Tumor antigens are cell surface molecules that are differentially expressed in tumor cells relative to normal cells. Tumor antigens distinguish tumor cells immunologically from normal cells and provide diagnostic and therapeutic targets for human cancers (Takagi, S. et al. (1995) *Int. J. Cancer* 61: 706-715; Liu, E. et al. (1992) *Oncogene* 7: 1027-1032). For example, the biliary glycoprotein-encoding gene is a member of the human carcinoembryonic antigen family, which are important tumor markers for colorectal carcinomas (Hammarstrom, S. (1999) *Semin. Cancer Bio.* 9:67-81). Another example is the neuron and testis specific protein Ma1, a marker for paraneoplastic neuronal disorders (Dalmau, J. et al. (1999) *Brain* 122:27-39).

Other types of cell surface antigens include those identified on leukocytic cells of the immune system. These antigens have been identified using systematic, monoclonal antibody (mAb)-based "shot gun" techniques. These techniques have resulted in the production of hundreds of mAbs directed against unknown cell surface leukocytic antigens. These antigens have been grouped into "clusters of differentiation" based on common immunocytochemical localization patterns in various differentiated and undifferentiated leukocytic cell types. Antigens in a given cluster are presumed to identify a single cell surface protein and are assigned a "cluster of differentiation" or "CD" designation. Some of the genes encoding proteins identified by CD antigens have been cloned and verified by standard molecular biology techniques. CD antigens have been characterized as both transmembrane proteins and cell surface proteins anchored to the plasma membrane via covalent attachment to fatty acid-containing glycolipids such as glycosylphosphatidylinositol (GPI). (Reviewed in Barclay, A. N. et al. (1995) *The Leucocyte Antigen Facts Book*, Academic Press, San Diego, CA, pp. 17-20.)

The TM cell surface glycoprotein CD69 is an early activation antigen of T lymphocytes. CD69 is homologous to members of a supergene family of type II integral membrane proteins having C-type lectin domains. Although the precise functions of the CD-69 antigen is not known, evidence suggests that these proteins transmit mitogenic signals across the plasma membrane and are up-regulated in response to lymphocyte activation (Hamann, J. et. al. (1993) J. Immunol. 150:4920-4927).

Macrophages are involved in functions including clearance of senescent or apoptotic cells, cytokine production, hemopoiesis, bone resorption, antigen transport, and neuroendocrine regulation. These diverse roles are influenced by specialized macrophage plasma membrane proteins. The murine macrophage restricted C-type lectin is a type II integral membrane protein expressed exclusively in macrophages. The strong expression of this protein in bone marrow suggests a hemopoietic function, while the lectin domain suggests it may be involved in cell-cell recognition (Balch, S. G. et al. (1998) J. Biol. Chem. 273:18656-18664).

The surface of red blood cells is populated with characteristic glycoproteins, such as the major sialoglycoproteins glycophorin A and B. Red blood cells lacking either glycophorin A or B are resistant to infection with the malaria parasite *Plasmodium falciparum* (OMIM Entry 111300 Blood Group-MN Locus). White blood cells also possess characteristic surface glycoproteins, such as the plasma cell glycoprotein-1 (PC-1). PC-1 is expressed on the surface of plasma cells, which are terminally differentiated, antibody-secreting B-lymphocytes. The extracellular domain of PC-1 has nucleotide phosphodiesterase (pyrophosphatase) activity (Funakoshi, I. et al. (1992) Arch. Biochem. Biophys. 295:180-187). Phosphodiesterase activity is associated with the hydrolytic removal of nucleotide subunits from oligonucleotides. Although the precise physiological role of PC-1 is not clear, increased PC-1 phosphodiesterase activity has been correlated with insulin resistance in patients with noninsulin-dependent diabetes mellitus, with abnormalities of bone mineralization and calcification, and with defects in renal tubule function. In addition, it appears that hPC-1 and mPC-1 are members of a multigene family of transmembrane phosphodiesterases with extracellular active sites. These enzymes may play a role in regulating the concentration of pharmacologically active extracellular compounds such as adenosine or other nucleotide derivatives in a variety of tissues and cell types. (Reviewed in Goding, J. W. et al. (1998) Immunol. Rev. 161:11-26.)

Peripheral and Anchored Membrane Proteins

Some membrane proteins are not membrane-spanning but are attached to the plasma membrane via membrane anchors or interactions with integral membrane proteins. Membrane anchors are covalently joined to a protein post-translationally and include such moieties as prenyl, myristyl, and glycosylphosphatidyl inositol (GPI) groups. Membrane localization of peripheral and

anchored proteins is important for their function in processes such as receptor-mediated signal transduction. For example, prenylation of Ras is required for its localization to the plasma membrane and for its normal and oncogenic functions in signal transduction.

The pancortins are a group of four glycoproteins which are predominantly expressed in the cerebral cortex of adult rodents. Immunological localization indicates that the pancortins are endoplasmic reticulum anchored proteins. The pancortins share a common sequence in the middle of their structure, but have alternative sequences at both ends due to differential promoter usage and alternative splicing. Each pancortin appears to be differentially expressed and may perform different functions in the brain (Nagano, T. et al. (1998) Mol. Brain Res. 53:13-23).

The discovery of new membrane associated proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative, autoimmune/inflammatory, neurological and gastrointestinal disorders.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, membrane associated proteins, referred to collectively as "MEMAP" and individually as "MEMAP-1," "MEMAP-2," "MEMAP-3," "MEMAP-4," "MEMAP-5," "MEMAP-6," "MEMAP-7," "MEMAP-8," "MEMAP-9," "MEMAP-10," "MEMAP-11," "MEMAP-12," "MEMAP-13," "MEMAP-14," "MEMAP-15," "MEMAP-16," "MEMAP-17," "MEMAP-18," "MEMAP-19," "MEMAP-20," "MEMAP-21," "MEMAP-22," "MEMAP-23," "MEMAP-24," "MEMAP-25," "MEMAP-26," "MEMAP-27," "MEMAP-28," "MEMAP-29," "MEMAP-30," "MEMAP-31," "MEMAP-32," "MEMAP-33," "MEMAP-34," "MEMAP-35," "MEMAP-36," and "MEMAP-37." In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-37.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group

consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-37. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:38-74.

5 Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37,
10 c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

15 The invention also provides a method for producing a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37,
20 c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

25 Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence
30 selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37.

The invention further provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, b) a naturally occurring polynucleotide sequence having at least
35 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID

NO:38-74, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a composition comprising an effective amount of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and a pharmaceutically acceptable excipient. In one embodiment, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. The invention additionally provides a

method of treating a disease or condition associated with decreased expression of functional MEMAP, comprising administering to a patient in need of such treatment the composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional MEMAP, comprising administering to a patient in need of such treatment the composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional MEMAP, comprising administering to a patient in need of such treatment the composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. The method comprises a)

combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-37. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:38-74, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, ii) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, ii) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:38-74, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence

- complementary to ii), and v) an RNA equivalent of i)-iv). Alternatively, the target polynucleotide comprises a fragment of the above polynucleotide sequence; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

BRIEF DESCRIPTION OF THE TABLES

- Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding MEMAP.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of MEMAP.

- Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding MEMAP were isolated.

- Table 5 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

- Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

- It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

- Unless defined otherwise, all technical and scientific terms used herein have the same

5

10

15

20

25

30

having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence.

Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of MEMAP. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of MEMAP either by directly interacting with MEMAP or by acting on components of the biological pathway in which MEMAP participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind MEMAP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified

sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic MEMAP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding MEMAP or fragments of MEMAP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (PE Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
5	Asp	Asn, Glu
	Cys	Ala, Ser
	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
10	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
15	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
20	Tyr	His, Phe, Trp
	Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide.

Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

A "fragment" is a unique portion of MEMAP or the polynucleotide encoding MEMAP which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino

acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:38-74 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:38-74, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:38-74 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:38-74 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:38-74 and the region of SEQ ID NO:38-74 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-37 is encoded by a fragment of SEQ ID NO:38-74. A fragment of SEQ ID NO:1-37 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-37. For example, a fragment of SEQ ID NO:1-37 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-37. The precise length of a fragment of SEQ ID NO:1-37 and the region of SEQ ID NO:1-37 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full-length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full-length" polynucleotide sequence encodes a "full-length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS

8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

- 5 Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bf2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST
- 10 programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

- 20 *Penalty for mismatch: -2*

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

- 25 *Filter: on*

- Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous
- 30 nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

- Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes
- 35 in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid

sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (Apr-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for chromosome replication, segregation and maintenance.

The term "humanized antibody" refers to an antibody molecule in which the amino acid

sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A

hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

5 The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect
10 cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of MEMAP which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of MEMAP which is useful in any of the antibody production methods disclosed herein or known in
15 the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

20 The term "modulate" refers to a change in the activity of MEMAP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of MEMAP.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or
25 synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding
30 sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition.

35 PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript

0965529-09601
109260-6255560

elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an MEMAP may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of MEMAP.

"Probe" refers to nucleic acid sequences encoding MEMAP, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al. (1987) Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3

primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear

sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding MEMAP, or fragments thereof, or MEMAP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants, and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook, J. et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence

identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human membrane associated proteins (MEMAP), the polynucleotides encoding MEMAP, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative, autoimmune/inflammatory, neurological and gastrointestinal disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding MEMAP. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each MEMAP were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. In some cases, GenBank sequence identifiers are also shown in column 5. The Incyte clones and GenBank cDNA sequences, where indicated, in column 5 were used to assemble the consensus nucleotide sequence of each MEMAP and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding MEMAP. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:38-74 and to distinguish between SEQ ID NO:38-74 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express MEMAP as a fraction of total tissues expressing MEMAP. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing MEMAP as a fraction of total tissues expressing MEMAP. Column 5 lists the vectors used to subclone each cDNA library. Of particular note is the expression of SEQ ID NO:41, SEQ ID NO:48, and SEQ ID NO:56 in nervous tissues, of SEQ ID NO:52, SEQ ID NO:65, and SEQ ID NO:74 in gastrointestinal

tissues, and of SEQ ID NO:55 in hematopoietic/immune tissues.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding MEMAP were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:38 maps to chromosome 4 within the interval from 77.9 to 86.0 centiMorgans, to chromosome 6 within the interval from 132.7 to 144.4 centiMorgans, and to chromosome 14 within the interval from 89.4 to 103.7 centiMorgans. The interval on chromosome 4 from 77.9 to 86.0 centiMorgans also contains a gene associated with deoxycytidine kinase deficiency. The interval on chromosome 6 from 132.7 to 144.4 centiMorgans also contains genes associated with peroxisomal disorders and leukemia. The interval on chromosome 14 from 89.4 to 103.7 centiMorgans also contains genes associated with spinocerebellar ataxia and protease inhibitor deficiencies. SEQ ID NO:39 maps to chromosome 2 within the interval from 236.2 to 269.5 centiMorgans, and to the X chromosome within the interval from 94.4 to 97.4 centiMorgans. The interval on chromosome 2 from 236.2 to 269.5 centiMorgans also contains genes associated with Crigler-Najjar syndrome, Oguchi disease, and oxalosis I. The interval on the X chromosome from 94.4 to 97.4 centiMorgans also contains genes associated with Charcot-Marie tooth disease, X-linked severe combined immunodeficiency, alpha thalassemia/mental retardation syndrome, Menkes' syndrome, and choroideremia. SEQ ID NO:42 maps to chromosome 1 within the interval from 218.2 to 232.0 centiMorgans. This interval also contains genes associated with familial hypertrophic cardiomyopathy, malignant hyperthermia, and hypokalemic periodic paralysis. SEQ ID NO:44 maps to chromosome 7 within the interval from 136.4 to 145.8 centiMorgans, to chromosome 14 within the interval from 28.0 to 32.9 centiMorgans, and to chromosome 14 within the interval from 71.5 to 73.7 centiMorgans. The interval on chromosome 7 from 136.4 to 145.8 centiMorgans also contains genes associated with diphosphoglycerate mutase deficiency. SEQ ID NO:60 maps to chromosome 7 within the interval from 167.6 to 184.0 centiMorgans, and to chromosome 14 within the interval from 50.0 to 59.0 centiMorgans. SEQ ID NO:63 maps to chromosome 8 within the interval from 101.0 to 125.8 centiMorgans, and to chromosome 8 within the interval from 132.4 to 135.1 centiMorgans. SEQ ID NO:67 maps to chromosome 4 within the interval from 145.3 to 146.4 centiMorgans.

The invention also encompasses MEMAP variants. A preferred MEMAP variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the MEMAP amino acid sequence, and which contains at least one functional or structural characteristic of MEMAP.

The invention also encompasses polynucleotides which encode MEMAP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected

from the group consisting of SEQ ID NO:38-74, which encodes MEMAP. The polynucleotide sequences of SEQ ID NO:38-74, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

5 The invention also encompasses a variant of a polynucleotide sequence encoding MEMAP. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding MEMAP. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID
10 NO:38-74 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:38-74. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of MEMAP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the
15 genetic code, a multitude of polynucleotide sequences encoding MEMAP, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the
20 polynucleotide sequence of naturally occurring MEMAP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode MEMAP and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring MEMAP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding
25 MEMAP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding MEMAP and its derivatives without altering the encoded amino acid
30 sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode MEMAP and MEMAP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell
35 systems using reagents well known in the art. Moreover, synthetic chemistry may be used to

introduce mutations into a sequence encoding MEMAP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:38-74 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (PE Biosystems, Foster City CA), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (PE Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (PE Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding MEMAP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) *PCR Methods Applic.* 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) *Nucleic Acids Res.* 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) *PCR Methods Applic.* 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve

unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, PE Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode MEMAP may be cloned in recombinant DNA molecules that direct expression of MEMAP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express MEMAP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter MEMAP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such

as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Cramer, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of MEMAP, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding MEMAP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.) Alternatively, MEMAP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (PE Biosystems). Additionally, the amino acid sequence of MEMAP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, supra, pp. 28-53.)

In order to express a biologically active MEMAP, the nucleotide sequences encoding MEMAP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in

polynucleotide sequences encoding MEMAP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding MEMAP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding MEMAP and its initiation

5 codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic.

10 The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding MEMAP and appropriate transcriptional and translational

15 control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

20 A variety of expression vector/host systems may be utilized to contain and express sequences encoding MEMAP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower

25 mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509; Bitter, G.A. et al. (1987) *Methods Enzymol.* 153:516-544; Scorer, C.A. et al. (1994) *BioTechnology* 12:181-184; Engelhard, E.K. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3224-3227; Sandig, V. et al. (1996) *Hum. Gene Ther.* 7:1937-

30 1945; Takamatsu, N. (1987) *EMBO J.* 6:307-311; Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al. (1984) *Science* 224:838-843; Winter, J. et al. (1991) *Results Probl. Cell Differ.* 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci. USA* 81:3655-3659; and Harrington, J.J. et al. (1997) *Nat. Genet.* 15:345-355.) Expression vectors derived from retroviruses,

35 adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for

delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) *Cancer Gen. Ther.* 5(6):350-356; Yu, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(13):6340-6344; Buller, R.M. et al. (1985) *Nature* 317(6040):813-815; McGregor, D.P. et al. (1994) *Mol. Immunol.* 31(3):219-226; and Verma, I.M. and N. Somia (1997) *Nature* 389:239-242.)

- 5 The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding MEMAP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding MEMAP can be achieved using a multifunctional *E. coli* vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPTORT 10 plasmid (Life Technologies). Ligation of sequences encoding MEMAP into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for *in vitro* transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) *J. Biol.* 15 *Chem.* 264:5503-5509.) When large quantities of MEMAP are needed, e.g. for the production of antibodies, vectors which direct high level expression of MEMAP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of MEMAP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH 20 promoters, may be used in the yeast *Saccharomyces cerevisiae* or *Pichia pastoris*. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, *supra*; Bitter, *supra*; and Scorer, *supra*.)

Plant systems may also be used for expression of MEMAP. Transcription of sequences 25 encoding MEMAP may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J.* 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, *supra*; Broglie, *supra*; and Winter, *supra*.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated 30 transfection. (See, e.g., *The McGraw Hill Yearbook of Science and Technology* (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding MEMAP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite 35 leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to

0965529-092601

obtain infective virus which expresses MEMAP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

- 5 Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)
- 10 For long term production of recombinant proteins in mammalian systems, stable expression of MEMAP in cell lines is preferred. For example, sequences encoding MEMAP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in
- 15 enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

- Any number of selection systems may be used to recover transformed cell lines. These
- 20 include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk⁻* and *aprt⁻* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat*
- 25 confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins
- 30 (GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

- Although the presence/absence of marker gene expression suggests that the gene of interest is
- 35 also present, the presence and expression of the gene may need to be confirmed. For example, if the

sequence encoding MEMAP is inserted within a marker gene sequence, transformed cells containing sequences encoding MEMAP can be identified by the absence of marker gene function.

Alternatively, a marker gene can be placed in tandem with a sequence encoding MEMAP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding MEMAP and that express MEMAP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of MEMAP using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on MEMAP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding MEMAP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding MEMAP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding MEMAP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence

and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode MEMAP may be designed to contain signal sequences which direct secretion of MEMAP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding MEMAP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric MEMAP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of MEMAP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the MEMAP encoding sequence and the heterologous protein sequence, so that MEMAP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, *supra*, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled MEMAP may be achieved *in vitro* using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ³⁵S-methionine.

MEMAP of the present invention or fragments thereof may be used to screen for compounds that specifically bind to MEMAP. At least one and up to a plurality of test compounds may be screened for specific binding to MEMAP. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

- 5 In one embodiment, the compound thus identified is closely related to the natural ligand of MEMAP, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which MEMAP binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the
- 10 compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express MEMAP, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing MEMAP or cell membrane fractions which contain MEMAP are then contacted with a test compound and binding, stimulation, or inhibition of activity of either MEMAP
- 15 or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with MEMAP, either in solution or affixed to a solid support, and detecting the binding of MEMAP to the compound.

- 20 Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

- MEMAP of the present invention or fragments thereof may be used to screen for compounds
- 25 that modulate the activity of MEMAP. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for MEMAP activity, wherein MEMAP is combined with at least one test compound, and the activity of MEMAP in the presence of a test compound is compared with the activity of MEMAP in the absence of the test compound. A change in the activity of MEMAP in the presence of the test compound is
- 30 indicative of a compound that modulates the activity of MEMAP. Alternatively, a test compound is combined with an in vitro or cell-free system comprising MEMAP under conditions suitable for MEMAP activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of MEMAP may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

- 35 In another embodiment, polynucleotides encoding MEMAP or their mammalian homologs

may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent No. 5,175,383 and U.S. Patent No. 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding MEMAP may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding MEMAP can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding MEMAP is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress MEMAP, e.g., by secreting MEMAP in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of MEMAP and membrane associated proteins. In addition, the expression of MEMAP is closely associated with neurological and gastrointestinal tissues, cancer, cell proliferation, and inflammation/trauma. Therefore, MEMAP appears to play a role in cell proliferative, autoimmune/inflammatory, neurological and gastrointestinal disorders. In the treatment

of disorders associated with increased MEMAP expression or activity, it is desirable to decrease the expression or activity of MEMAP. In the treatment of disorders associated with decreased MEMAP expression or activity, it is desirable to increase the expression or activity of MEMAP.

Therefore, in one embodiment, MEMAP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MEMAP. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis,

encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; and a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha₁-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas.

In another embodiment, a vector capable of expressing MEMAP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MEMAP including, but not limited to, those described above.

In a further embodiment, a composition comprising a substantially purified MEMAP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MEMAP including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of MEMAP may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MEMAP including, but not limited to, those listed above.

In a further embodiment, an antagonist of MEMAP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of MEMAP. Examples of such

disorders include, but are not limited to, those cell proliferative, autoimmune/inflammatory, neurological and gastrointestinal disorders described above. In one aspect, an antibody which specifically binds MEMAP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express MEMAP.

5 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding MEMAP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of MEMAP including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

15 An antagonist of MEMAP may be produced using methods which are generally known in the art. In particular, purified MEMAP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind MEMAP. Antibodies to MEMAP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

20 For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with MEMAP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to MEMAP have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of MEMAP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

35 Monoclonal antibodies to MEMAP may be prepared using any technique which provides for

the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:2026-2030; and
 5 Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda,
 10 S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce MEMAP-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) *Proc. Natl. Acad. Sci. USA* 88:10134-10137.)

15 Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3833-3837; Winter, G. et al. (1991) *Nature* 349:293-299.)

Antibody fragments which contain specific binding sites for MEMAP may also be generated.
 20 For example, such fragments include, but are not limited to, F(ab)₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab)₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) *Science* 246:1275-1281.)

25 Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between MEMAP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies
 30 reactive to two non-interfering MEMAP epitopes is generally used, but a competitive binding assay may also be employed (Pound, *supra*).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for MEMAP. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of MEMAP-antibody complex
 35 divided by the molar concentrations of free antigen and free antibody under equilibrium conditions.

006529-02601

The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple MEMAP epitopes, represents the average affinity, or avidity, of the antibodies for MEMAP. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular MEMAP epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the MEMAP-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of MEMAP, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of MEMAP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al., supra.)

In another embodiment of the invention, the polynucleotides encoding MEMAP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding MEMAP. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding MEMAP. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other

systems known in the art. (See, e.g., Rossi, J.J. (1995) Br. Med. Bull. 51(1):217-225; Boado, R.J. et al. (1998) J. Pharm. Sci. 87(11):1308-1315; and Morris, M.C. et al. (1997) Nucleic Acids Res. 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding MEMAP may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in MEMAP expression or regulation causes disease, the expression of MEMAP from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in MEMAP are treated by constructing mammalian expression vectors encoding MEMAP and introducing these vectors by mechanical means into MEMAP-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J.-L. and H. Récipon (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of MEMAP include, but are not limited to, the pCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). MEMAP may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus

09965529 092601

(RSV), SV40 virus, thymidine kinase (TK), or β -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Gossen, M. et al. (1995) *Science* 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) *Curr. Opin. Biotechnol.* 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the
 5 ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and H.M. Blau, supra)), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding MEMAP from a normal individual.

- Commercially available liposome transformation kits (e.g., the PERFECT LIPID
 10 TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) *Virology* 52:456-467), or by electroporation (Neumann, E. et al. (1982) *EMBO J.* 1:841-845). The introduction of DNA to primary cells requires modification of
 15 these standardized mammalian transfection protocols.

- In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to MEMAP expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding MEMAP under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive
 20 element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for
 25 receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) *J. Virol.* 61:1647-1650; Bender, M.A. et al. (1987) *J. Virol.* 61:1639-1646; Adam, M.A. and A.D. Miller (1988) *J. Virol.* 62:3802-3806; Dull, T. et al. (1998) *J. Virol.* 72:8463-8471; Zufferey, R. et al. (1998) *J. Virol.* 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant")
 30 discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4⁺ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) *J. Virol.* 71:7020-7029; Bauer, G. et al. (1997) *Blood* 89:2259-2267; Bonyhadi, M.L. (1997) *J. Virol.* 71:4707-4716;
 35 Ranga, U. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:1201-1206; Su, L. (1997) *Blood* 89:2283-

2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding MEMAP to cells which have one or more genetic abnormalities with respect to the expression of MEMAP. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) *Transplantation* 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) *Annu. Rev. Nutr.* 19:511-544; and Verma, I.M. and N. Somia (1997) *Nature* 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding MEMAP to target cells which have one or more genetic abnormalities with respect to the expression of MEMAP. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing MEMAP to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) *Exp. Eye Res.* 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) *J. Virol.* 73:519-532 and Xu, H. et al. (1994) *Dev. Biol.* 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding MEMAP to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) *Curr. Opin. Biotechnol.* 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid

proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for MEMAP into the alphavirus genome in place of the capsid-coding region results in the production of a large number of MEMAP-coding RNAs and the synthesis of high levels of MEMAP in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) *Virology* 228:74-83). The wide host range of alphaviruses will allow the introduction of MEMAP into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding MEMAP.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding MEMAP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding MEMAP. Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased MEMAP expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding MEMAP may be therapeutically useful, and in the treatment of disorders associated with decreased MEMAP expression or activity, a compound which specifically promotes expression of the polynucleotide encoding MEMAP may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a

polynucleotide encoding MEMAP is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding MEMAP are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding MEMAP. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruce, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruce, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such compositions may consist of MEMAP, antibodies to MEMAP, and mimetics, agonists, antagonists, or inhibitors of MEMAP.

The compositions utilized in this invention may be administered by any number of routes

including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Compositions for pulmonary administration may be prepared in liquid or dry powder form.

- 5 These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, 10 J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

- 15 Specialized forms of compositions may be prepared for direct intracellular delivery of macromolecules comprising MEMAP or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, MEMAP or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to 20 transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

- For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration 25 range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

- A therapeutically effective dose refers to that amount of active ingredient, for example MEMAP or fragments thereof, antibodies of MEMAP, and agonists, antagonists or inhibitors of MEMAP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be 30 determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED₅₀ (the dose therapeutically effective in 50% of the population) or LD₅₀ (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD₅₀/ED₅₀ ratio. Compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are 35 used to formulate a range of dosage for human use. The dosage contained in such compositions is

preferably within a range of circulating concentrations that includes the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μ g to 100,000 μ g, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind MEMAP may be used for the diagnosis of disorders characterized by expression of MEMAP, or in assays to monitor patients being treated with MEMAP or agonists, antagonists, or inhibitors of MEMAP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for MEMAP include methods which utilize the antibody and a label to detect MEMAP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring MEMAP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of MEMAP expression. Normal or standard values for MEMAP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to MEMAP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of MEMAP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding MEMAP may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of MEMAP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of MEMAP, and to monitor regulation of MEMAP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding MEMAP or closely related molecules may be used to identify nucleic acid sequences which encode MEMAP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding MEMAP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the MEMAP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:38-74 or from genomic sequences including promoters, enhancers, and introns of the MEMAP gene.

Means for producing specific hybridization probes for DNAs encoding MEMAP include the cloning of polynucleotide sequences encoding MEMAP or MEMAP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding MEMAP may be used for the diagnosis of disorders associated with expression of MEMAP. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis,

anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, 5 erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, 10 ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, 15 progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the 20 nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, 25 endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; and a gastrointestinal disorder such as dysphagia, peptic esophagitis, 30 esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, 35 ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome,

colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha₁-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas. The polynucleotide sequences encoding MEMAP may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered MEMAP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding MEMAP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding MEMAP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding MEMAP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of MEMAP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding MEMAP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several

days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding MEMAP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding MEMAP, or a fragment of a polynucleotide complementary to the polynucleotide encoding MEMAP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding MEMAP may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding MEMAP are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed *in silico* SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of MEMAP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; Duplaa, C.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described in Seilhamer, J.J. et al., "Comparative Gene Transcript Analysis," U.S. Patent No. 5,840,484, incorporated herein by reference. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson (2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are

separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, supra). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for MEMAP to quantify the levels of MEMAP expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendoze, L.G. et al. (1999) *Biotechniques* 27:778-788). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J. Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing

the amino acid residues of the individual proteins and comparing these partial sequences to the polypeptides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding MEMAP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, e.g., Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding MEMAP on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the

region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, MEMAP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between MEMAP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with MEMAP, or fragments thereof, and washed. Bound MEMAP is then detected by methods well known in the art. Purified MEMAP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding MEMAP specifically compete with a test compound for binding MEMAP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with MEMAP.

In additional embodiments, the nucleotide sequences which encode MEMAP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific

embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/149,641 and U.S. Ser. No. 60/164,203 are hereby expressly incorporated
5 by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some
10 tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA
15 purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA
20 purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units
25 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs
30 were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Genomics, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, 5 QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal 10 cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

15 Inocyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (PE Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents 20 provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (PE Biosystems) in conjunction with standard ABI protocols and base 25 calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed 30 using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their 35 entirety, and the fourth column presents, where applicable, the scores, probability values, and other

parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default
 5 parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST,
 10 dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled
 15 into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying
 20 against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene
 families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID
 25 NO:38-74. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs
 30 from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or
 35 similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum} \{ \text{length}(\text{Seq. 1}), \text{length}(\text{Seq. 2}) \}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding MEMAP occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Chromosomal Mapping of MEMAP Encoding Polynucleotides

The cDNA sequences which were used to assemble SEQ ID NO:38-74 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:38-74 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location.

The genetic map locations of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:60, SEQ ID NO:63, and SEQ ID NO:67 are described in The Invention as ranges, or intervals, of human chromosomes. More than one map location is reported for SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:60, and SEQ ID NO:63, indicating that previously mapped sequences having similarity, but not complete identity, to SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:60, and SEQ ID NO:63 were assembled into their respective clusters. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Diseases associated with the public and Incyte sequences located within the indicated intervals are also reported in the Invention where applicable. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site (<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

VI. Extension of MEMAP Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:38-74 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68 °C to about 72 °C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$, and β -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94 °C, 3 min; Step 2: 94 °C, 15 sec; Step 3: 60 °C, 1 min; Step 4: 68 °C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68 °C, 5 min; Step 7: storage at 4 °C. In the

alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems).

In like manner, the polynucleotide sequences of SEQ ID NO:38-74 are used to obtain 5' regulatory sequences using the procedure above, along with oligonucleotides designed for such extension, and an appropriate genomic library.

VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:38-74 are employed to screen cDNAs,

genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [5 5 γ -³²P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10⁷ counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases:

10 Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate.

15 Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

VIII. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, supra),

20 mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Schena (1999), supra). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding

25 procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) *Science* 270:467-470; Shalon, D. et al. (1996) *Genome Res.* 6:639-645; Marshall, A. and J. Hodgson (1998) *Nat. Biotechnol.* 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may

30 comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a

35 fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser

desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

5 Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)⁺ RNA is purified using the oligo-(dT) cellulose method. Each poly(A)⁺ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ μ l oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/ μ l RNase inhibitor, 500 μ M dATP, 500 μ M dGTP, 500 μ M dTTP, 40 μ M dCTP, 40 μ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)⁺ RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)⁺ RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μ l 5X SSC/0.2% SDS.

20 Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 μ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 μ l of the array element DNA, at an average

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60 °C followed by washes in 0.2% SDS and distilled water as before.

Hybridization reactions contain 9 μ l of sample mixture consisting of 0.2 μ g each of Cy3 and
10 Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample
mixture is heated to 65 $^{\circ}$ C for 5 minutes and is aliquoted onto the microarray surface and covered
with an 1.8 cm² coverslip. The arrays are transferred to a waterproof chamber having a cavity just
slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the
addition of 140 μ l of 5X SSC in a corner of the chamber. The chamber containing the arrays is
15 incubated for about 6.5 hours at 60 $^{\circ}$ C. The arrays are washed for 10 min at 45 $^{\circ}$ C in a first wash
buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45 $^{\circ}$ C in a second wash buffer (0.1X
SSC), and dried.

Reporter-labeled hybridization complexes are detected with a microscope equipped with an
20 Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines
at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is
focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide
containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-
scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a
25 resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

67

location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the

two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RT1-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

IX. Complementary Polynucleotides

Sequences complementary to the MEMAP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring MEMAP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of MEMAP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the MEMAP-encoding transcript.

X. Expression of MEMAP

Expression and purification of MEMAP is achieved using bacterial or virus-based expression systems. For expression of MEMAP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express MEMAP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of MEMAP in eukaryotic cells is achieved by infecting

insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding MEMAP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (SF9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, MEMAP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from MEMAP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified MEMAP obtained by these methods can be used directly in the assays shown in Examples XI and XV.

XI. Demonstration of MEMAP Activity

MEMAP activity is demonstrated using a generic immunoblotting strategy or through a MEMAP-specific activity assay as outlined below. As a general approach, cell lines or tissues transformed with a vector containing MEMAP coding sequences can be assayed for MEMAP activity by immunoblotting. Transformed cells are denatured in SDS in the presence of β -mercaptoethanol, nucleic acids are removed by ethanol precipitation, and proteins are purified by acetone precipitation. Pellets are resuspended in 20 mM Tris buffer at pH 7.5 and incubated with Protein G-Sepharose pre-coated with an antibody specific for MEMAP. After washing, the Sepharose beads are boiled in electrophoresis sample buffer, and the eluted proteins subjected to SDS-PAGE. Proteins are transferred from the SDS-PAGE gel to a membrane for immunoblotting, and the MEMAP activity is assessed by visualizing and quantifying bands on the blot using antibody specific for MEMAP as the primary antibody and 125 I-labeled IgG specific for the primary antibody as the secondary antibody.

A specific assay for MEMAP activity measures the expression of MEMAP on the cell surface. cDNA encoding MEMAP is transfected into a mammalian (non-human) cell line. Cell

surface proteins are labeled with biotin as described in de la Fuente, M.A.. et al. ((1997) Blood 90:2398-2405). Immunoprecipitations are performed using MEMAP-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The ratio of labeled immunoprecipitant to unlabeled immunoprecipitant is proportional to the amount of MEMAP expressed on the cell surface.

In an alternative specific assay, MEMAP transport activity is assayed by measuring uptake of labeled substrates into *Xenopus laevis* oocytes. Oocytes at stages V and VI are injected with MEMAP mRNA (10 ng per oocyte) and incubated for 3 days at 18°C in OR2 medium (82.5mM NaCl, 2.5 mM KCl, 1mM CaCl₂, 1mM MgCl₂, 1mM Na₂HPO₄, 5 mM Hepes, 3.8 mM NaOH, 50µg/ml gentamycin, pH 7.8) to allow expression of MEMAP protein. Oocytes are then transferred to standard uptake medium (100mM NaCl, 2 mM KCl, 1mM CaCl₂, 1mM MgCl₂, 10 mM Hepes/Tris pH 7.5). Uptake of various substrates (e.g., amino acids, sugars, drugs, and neurotransmitters) is initiated by adding a ³H substrate to the oocytes. After incubating for 30 minutes, uptake is terminated by washing the oocytes three times in Na⁺-free medium, measuring the incorporated ³H, and comparing with controls. MEMAP activity is proportional to the level of internalized ³H substrate.

XII. Functional Assays

MEMAP function is assessed by expressing the sequences encoding MEMAP at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10 µg of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 µg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as

measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) *Flow Cytometry*, Oxford, New York NY.

The influence of MEMAP on gene expression can be assessed using highly purified
 5 populations of cells transfected with sequences encoding MEMAP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in
 10 the art. Expression of mRNA encoding MEMAP and other genes of interest can be analyzed by northern analysis or microarray techniques.

XIII. Production of MEMAP Specific Antibodies

MEMAP substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to
 15 immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the MEMAP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well
 20 described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (PE Biosystems) using Fmoc chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-
 25 KLH complex in complete Freund's adjuvant. Resulting antisera are tested for anti-peptide and anti-MEMAP activity by, for example, binding the peptide or MEMAP to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIV. Purification of Naturally Occurring MEMAP Using Specific Antibodies

Naturally occurring or recombinant MEMAP is substantially purified by immunoaffinity
 30 chromatography using antibodies specific for MEMAP. An immunoaffinity column is constructed by covalently coupling anti-MEMAP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing MEMAP are passed over the immunoaffinity column, and the column is
 35 washed under conditions that allow the preferential absorbance of MEMAP (e.g., high ionic strength

09965529-092601

buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/MEMAP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and MEMAP is collected.

XV. Identification of Molecules Which Interact with MEMAP

MEMAP, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled MEMAP, washed, and any wells with labeled MEMAP complex are assayed. Data obtained using different concentrations of MEMAP are used to calculate values for the number, affinity, and association of MEMAP with the candidate molecules.

Alternatively, molecules interacting with MEMAP are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, *Nature* 340:245-246), or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

MEMAP may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.